

Patient MRN ---	Patient Name ---, ---	Birth Date ---	Sex Female	Strata Case ---
Client Specimen ID ---	Specimen Site Lung, RII	Part ---	Date of Collection ---	Date Received ---
Client ---	Ordering Physician ---			

• Molecular Diagnosis and Tissue Specimen Information Determined at time of testing

Cancer Type	Tumor Content
Breast Cancer	80%
Cancer Subtype	Surface Area
Breast Invasive Carcinoma, NOS	5mm ²
Receptor Status	
ER: Positive, PR: Negative, HER2: Low, ERBB2 copy number: 2.2	

• Pertinent Biomarker Summary

Strata Select	
Genomic Alterations	MLL3 p.Q185* (38% VAF), PIK3CA p.R38H (37% VAF)
Negative Genomic Findings	BRCA1, BRCA2, ERBB2, ESR1, NTRK1, NTRK2, NTRK3, RET
Genomic Signatures	Microsatellite Stable, TMB Low (3 muts/Mb)
Immunotherapy Response Score	IRS Low
Strata RNA Supplement	
Gene Expression	ERBB2 (HER2) Low, ESR1 (ER) Positive, TACSTD2 (Trop2) High

• Therapy Associations

Biomarker Results	Predicted Benefit	Therapies	Evidence Level
PIK3CA p.R38H	● Benefit	alpelisib + fulvestrant ^{2,L}	3
TROP2 RNA High	● Benefit	sacituzumab govitecan ^{L*}	1
HER2 IHC 1-2+ / HER2 RNA Low-Moderate, No HER2 IHC / HER2 RNA Low-Moderate	● Benefit	fam-trastuzumab deruxtecan-nxki ^{L*}	1
HER2 IHC 0+ / HER2 RNA Low-Moderate	● Benefit	fam-trastuzumab deruxtecan-nxki ^{L*}	4
IRS Low	● Less benefit	pembrolizumab, nivolumab, or atezolizumab ^L	3

Strata Oncology Testing Summary

Therapy Rationale. Alpelisib is approved in hormone receptor positive / ERBB2 negative breast cancer for a limited set of PIK3CA activating mutations that were assessed in the pivotal trial. Whether the above reported prioritized PIK3CA mutation confers sensitivity to alpelisib is not entirely clear, however a recent analysis of real-world alpelisib+fulvestrant treatment in patients with extended spectrum PIK3CA mutations showed modestly increased benefit compared to fulvestrant alone (median real world PFS 4.0 versus 2.5 months; PMID:36321996).

The Strata HER2 RNA expression status is consistent with the outside IHC status and supports benefit of fam-trastuzumab deruxtecan-nxki.

The sample has at least low level TROP2 expression, and thus similar expected sacituzumab govitecan benefit compared to unselected patients.

Across pan-solid tumor validation cohorts, IRS Ultra-Low patients have the shortest anti-PD-(L)1 monotherapy real world progression free survival (rwPFS) and overall survival.

For all therapeutically relevant biomarkers, the predicted benefit of listed therapies based on the following levels of evidence in the reported tumor type is shown: Level 1: FDA recognized; 2: Standard of care biomarker recommended per NCCN; 3: Compelling clinical evidence; 4: Compelling biologic evidence. "Less benefit" predicts less clinical benefit compared to unselected patients. "Trial" indicates therapies available through partnered clinical trials. For therapies, superscripts 1/2/L/M denote line of therapy (first, second, later, maintenance); * denotes a NCCN preferred therapy. For tumor types where PD-(L)1 monotherapy is considered Level 1 or 2 evidence in the absence of a biomarker (or by PD-L1 immunohistochemistry), that level of evidence is maintained if the combination of MSI/TMB/IRS supports PD-(L)1 monotherapy benefit.

Strata Oncology Testing Summary Description

The Strata Oncology Testing Summary integrates all testing results performed on the indicated specimen to highlight the most clinically relevant information. Please review the following individual test reports for additional information, which may be informative for patient management. Additional descriptions of testing summarized herein are provided in the individual test descriptions. PD-L1 IHC testing, when ordered, is performed and interpreted by Mayo Clinic Laboratories; see the PD-L1 IHC report for additional information.

Treatment Decisions

The selection of any treatment or clinical trial suggested herein resides within the discretion and judgment of the treating physician and patient. Strata testing identified biomarkers and relevant treatment associations are shown and described in the 'Therapy Associations' section based on the final reported tumor type using the levels of evidence shown in that legend. Listed treatments and levels of evidence may not be applicable to the patient's specific cancer based on incorrectly reported tumor type (most commonly due to information not provided in the submitted pathology report), prior therapies, impact of other biomarkers not assessed by Strata testing, and specific indications requiring combination with non-targeted therapies. Decisions on patient care should be based on the independent medical judgment of the treating physician based upon all available clinical info and should not be based solely on the information contained in the Strata Oncology Testing Summary.

No Warranty or Guarantee of Clinical Benefit

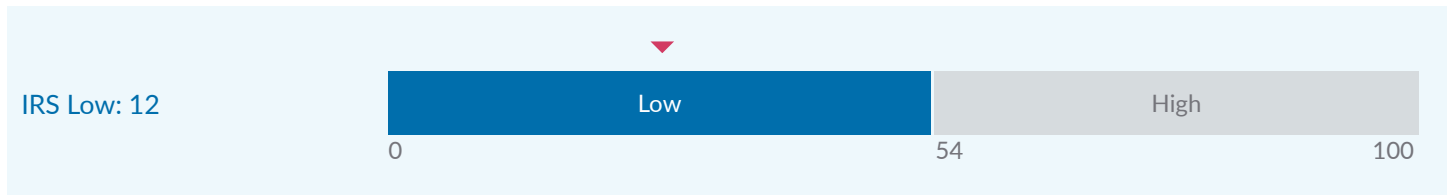
This Summary does not make any promise or guarantee that a particular therapy or clinical trial will be effective or helpful in the treatment of disease in any patient.

Patient MRN ---	Patient Name ---, ---	Birth Date ---	Sex Female	Strata Case ---
Client Specimen ID ---	Specimen Site Lung, RII	Part ---	Date of Collection ---	Date Received ---
Client ---	Ordering Physician ---			

• Molecular Diagnosis and Tissue Specimen Information Determined at time of testing

Cancer Type	Tumor Content
Breast Cancer	80%
Cancer Subtype	Surface Area
Breast Invasive Carcinoma, NOS	5mm²
Receptor Status	
ER: Positive, PR: Negative, HER2: Low, ERBB2 copy number: 2.2	

• Immunotherapy Response Score (IRS)



The Immunotherapy Response Score for this sample is **Low** (defined as a score <54).

IRS Low⁺ patients treated with anti-PD-(L)1 monotherapy have a shorter real world progression free survival (rwPFS)* compared to IRS High patients (<10 months vs >20 months).

⁺ This result should be considered along with other molecular results that may be associated with immunotherapy, including TMB and MSI. Given the rarity of TMB-H/IRS-L or MSI-H/IRS-L patients in the IRS validation studies, therapeutic decision making in these cases should be based on TMB and/or MSI status.

* Data presented is aggregate and pan-tumor. Real world progression free survival can vary by tumor type, line of therapy, age and gender.

1. Tomlins, S.A., Khazanov, N.A., Bulen, B.J. et al. Development and validation of an integrative pan-solid tumor predictor of PD-1/PD-L1 blockade benefit. Commun Med 3, 14 (2023). <https://doi.org/10.1038/s43856-023-00243-7>

2. Bulen BJ, Khazanov NA, Hovelson DH, et al. Validation of Immunotherapy Response Score as Predictive of Pan-solid Tumor Anti-PD-1/PD-L1 Benefit. Cancer Res Commun 3, 7 (2023) <https://doi.org/10.1158/2767-9764.CRC-23-0036>

• Genomic Signatures

Biomarker	Biomarker
MSS	TMB - Low
Microsatellite Stable	Mutations per MB: 3

- Prioritized Comprehensive Genomic Profiling Results

Biomarker

MLL3 p.Q185*

NM_170606.2:c.553C>T

Estimated variant allele frequency: 38%

Biomarker

PIK3CA p.R38H

NM_006218.2:c.113G>A

Estimated variant allele frequency: 37%

- Notes

- The specimen yielded DNA concentration less than 1ng/uL.

• Remaining Negative Comprehensive Genomic Profiling Results

The patient tested negative for all targeted genomic alterations in the following genes:

Prioritized hotspot mutations

AKT1	ALK	AR	ARAF	BRAF	CDK4	CTNNB1	EGFR	ERBB2	ERBB3	ERBB4
ESR1	EZH2	FGFR2	FGFR3	GNA11	GNAQ	HRAS	IDH1	JAK1	JAK2	JAK3
KIT	KRAS	MAP2K1	MAP2K2	MAP2K4	MAP2K7	MAPK1	MET	MTOR	MYD88	NRAS
NTRK1	PDGFRA	POLE	RAF1	RET	RIT1	ROS1	SF3B1	SMO	SPOP	TERT

Prioritized hotspot mutations or deleterious mutations

ATM	BRCA1	BRCA2	CDKN2A	MSH2	MSH6	PTEN	RB1	TP53		
-----	-------	-------	--------	------	------	------	-----	------	--	--

Gene amplifications

AKT1	AKT2	AKT3	ALK	AR	AURKA	AXL	BRAF	CCND1	CCND2	CCNE1
CDK4	CDK6	CDK8	CRKL	DEK	DNMT3A	EGFR	ERBB2	ERBB3	ERBB4	ESR1
FGFR1	FGFR2	FGFR3	FGFR4	FOXP4	HIF1A	HRAS	IGF1R	IL6ST	KDR	KIT
KRAS	MAP2K1	MAPK1	MCL1	MDM2	MDM4	MET	MYB	MYC	MYCL	MYCN
NRAS	NTRK1	NTRK3	PDGFRA	PDGFRB	PIK3C2B	PIK3CA	PIK3CB	PPARG	RAF1	RET
ROS1	UBR5									

Deep gene deletions

APC	ARID1A	ARID2	ATM	ATR	ATRX	BAP1	BCL10	BCL11B	BLM	BMPR1A
BRCA1	BRCA2	BRIP1	CDC73	CDH1	CDK12	CDKN2A	CDKN2B	CDKN2C	CEBPA	CHEK1
CHEK2	CIC	CTNNA1	CYLD	DAXX	DCC	DICER1	ERCC2	ERCC3	ERCC4	FAM123B
FANCA	FANCC	FANCD2	FANCF	FANCG	FAS	FBXW7	FH	FLCN	FOXO1	KDM5C
KDM6A	KEAP1	LRP1B	MAP3K7	MEN1	MLH1	MLL	MLL2	MLL3	MRE11A	MSH2
MSH6	MUTYH	NBN	NF1	NF2	NOTCH1	NOTCH2	NOTCH4	NSD1	PALB2	PARP1
PBRM1	PHOX2B	PIK3R1	PIK3R2	PMS1	PMS2	POT1	PPP2R1A	PRDM1	PTEN	PTPRD
PTPRT	RAD50	RB1	RUNX1	SDHA	SDHB	SDHC	SDHD	SETD2	SH2D1A	SMAD2
SMAD4	SMARCA4	SMARCB1	STK11	SUFU	TCF3	TCF7L2	TET1	TET2	TGFBR2	TNFAIP3
TNFRSF14	TOP1	TP53	TSC1	TSC2	VHL	WRN	WT1	XRCC2		

Gene fusions

AKT2	ALK	AXL	BRAF	COL6A3	CSF1	DDIT3	EGFR	ERBB2	ERBB4	ERG
ESR1	ETV1	ETV4	ETV5	EWSR1	FGFR1	FGFR2	FGFR3	FGR	FLI1	FLT3
FN1	FOXO1	FUS	JAK2	KRAS	MET	MYB	MYBL1	NCOA1	NCOA2	NF1
NOTCH1	NOTCH4	NR4A3	NRG1	NTRK1	NTRK2	NTRK3	NUTM1	PDGFB	PDGFRA	PDGFRB
PIK3CA	PPARG	PRKACA	PRKACB	PTEN	RAD51B	RAF1	RELA	RET	ROS1	RSPO2
RSPO3	S100A10	SSX1	SSX4	STAT6	TERT	TFE3	USP6	WWTR1	YWHAE	YY1

• No Calls

96.6% of tested positions yielded > 50x coverage. Specific positions not meeting usual coverage requirements are available upon request.

• Variants of Unknown Significance Non-synonymous, likely somatic

BCL11A | p.R713H

NM_022893.3

c.2138G>A | 37% VAF

CHEK1 | p.H293Y

NM_001274.5

c.877C>T | 54% VAF

DNMT3A | p.Q415Hfs*236

NM_022552.4

c.1245del | 45% VAF

These variants are included in case they are meaningful now or in the future. VAF values marked with asterisks are likely subclonal.

• DNA Genes

Prioritized mutations and high-confidence somatic variants of unknown significance are reported for the following 417 genes from a wide panel and a high depth (genes indicated in bold) panel. Copy number alterations (amplifications for oncogenes and deep deletions for tumor suppressors) are reported for underlined genes. Genes indicated with an asterisk have targeted coverage for reporting of pre-specified hotspot mutations from the high depth panel only.

ABL1	BCL6	CDKN2B	EPHA3	FLI1	IGF2R	LTK	MTR	<u>PALB2</u>	PRDM1	SETD2	TGM7
ABL2	BCL9	CDKN2C	EPHA7	FLT1	IKBKB	MAF	MTRR	PARP1	PRKAR1A	SF3B1	THBS1
ACVR2A	BCR	CEBPA	EPHB1	FLT3	IKBKE	MAFB	MUC1	PAX3	PRKDC	SGK1	TIMP3
ADAMTS20	BIRC2	CHEK1	EPHB4	FLT4	IKZF1	MAGEA1	MUTYH	PAX5	PSIP1	SH2D1A	TLR4
AFF1	BIRC3	CHEK2	EPHB6	FN1	IL2	MAGI1	MYB	PAX7	PTCH1	SMAD2	TLX1
AFF3	BIRC5	CIC	ERBB2	FOXL2	IL21R	MALT1	MYC	PAX8	PTEN	<u>SMAD4</u>	TNFAIP3
AKAP9	BLM	CKS1B	ERBB3	FOXO1	IL6ST	MAML2	MYCL	PBRM1	PTGS2	<u>SMARCA4</u>	TNFRSF14
AKT1	BLNK	CMPK1	ERBB4	FOXO3	IL7R	MAP2K1	MYCN	PBX1	PTPN11	<u>SMARCB1</u>	TNK2
<u>AKT2</u>	BMPR1A	COL1A1	ERCC1	FOXP1	ING4	MAP2K2	MYD88	PDE4DIP	PTPRD	SMO	TOP1
AKT3	BRAF	CRBN	ERCC2	FOXP4	IRF4	MAP2K4	MYH11	PDGFB	PTPRT	SMUG1	TP53
ALK	BRCA1	CREB1	ERCC3	FZR1	IRS2	MAP2K7*	MYH9	PDGFRA	RAD50	SOC3	TPR
<u>APC</u>	BRCA2	CREBBP	ERCC4	G6PD	ITGA10	MAP3K7	NBN	<u>PDGFRB</u>	RAF1	SOX11	TRIM24
AR	BRD3	CRKL	ERCC5	GATA1	ITGA9	MAPK1	NCOA1	PER1	RALGDS	SOX2	TRIM33
ARAF*	BRIP1	CRTC1	ERG	GATA2	ITGB2	MAPK8	NCOA2	PGAP3	RARA	SPOP*	TRIP11
ARID1A	BTK	CSF1R	ESR1	GATA3	ITGB3	MARK1	NCOA4	PHOX2B	RB1	SRC	TRRAP
ARID2	BUB1B	CSMD3	ETS1	GDNF	JAK1	MARK4	NF1	PIK3C2B	RECQL4	SSX1	TSC1
ARNT	CARD11	<u>CTNNA1</u>	ETV1	GNA11	JAK2	MBD1	NF2	PIK3CA	REL	STK11	TSC2
ASXL1	CASC5	CTNNB1	ETV4	GNAQ	JAK3	MCL1	NFE2L2	PIK3CB	RET	STK36	TSHR
ATF1	CBL	CYLD	EXT1	GNAS	JUN	MDM2	NFKB1	PIK3CD	RHOH	SUFU	UBR5
ATM	CCND1	CYP2C19	EXT2	GPR124	KAT6A	MDM4	NFKB2	PIK3CG	RIT1*	SYK	UGT1A1
ATR	CCND2	CYP2D6	EZH2	GRM8	KAT6B	MEN1	NIN	PIK3R1	RNASEL	SYNE1	USP9X
ATRX	<u>CCNE1</u>	DAXX	FAM123B	GUCY1A2	KDM5C	MET	NKX2-1	PIK3R2	RNF2	TAF1	VHL
AURKA	CD79A	DCC	FANCA	HCAR1	KDM6A	MITF	NLRP1	PIM1	RNF213	TAF1L	WAS
AURKB	CD79B	DDB2	FANCC	HIF1A	KDR	MLH1	NOTCH1	PKHD1	ROS1	TAL1	WHSC1
AURKC	CDC73	DDIT3	FANCD2	HLF	KEAP1	MLL	NOTCH2	PLAG1	RPS6KA2	TBX22	WRN
AXL	<u>CDH1</u>	DDR2	FANCF	HNF1A	KIT	MLL2	NOTCH4	PLCG1	RRM1	TCF12	WT1
BAI3	CDH11	DEK	FANCG	HOOK3	KLF6	MLL3	NPM1	PLEKHG5	RUNX1	TCF3	XPA
BAP1	CDH2	DICER1	FAS	HRAS	KRAS	MLLT10	NRAS	PML	RUNX1T1	TCF7L1	XPC
BCL10	CDH20	DNMT3A	FBXW7	HSP90AA1	LAMP1	MMP2	NSD1	PMS1	SAMD9	<u>TCF7L2</u>	XPO1
BCL11A	CDH5	DPYD	FGFR1	HSP90AB1	LCK	MN1	NTRK1	PMS2	SBDS	<u>TCL1A</u>	XRCC2
BCL11B	<u>CDK12</u>	DST	FGFR2	ICK	LIFR	MPL	NTRK3	POLE*	SDHA	TERT*	ZNF384
BCL2	CDK4	EGFR	FGFR3	IDH1	LPHN3	MRE11A	NUMA1	POT1	SDHB	TET1	ZNF521
BCL2L1	CDK6	EML4	FGFR4	IDH2	LPP	MSH2	NUP214	POU5F1	SDHC	TET2	
BCL2L2	CDK8	EP300	FH	IGF1R	LRP1B	MSH6	NUP98	PPARG	SDHD	TFE3	
BCL3	CDKN2A	EP400	FLCN	IGF2	LTF	MTOR	PAK3	PPP2R1A	SEPT9	TGFB2	

• RNA Genes

950 individual fusion isoforms involving the following 59 driver genes are targeted; additional fusion isoforms amplified from non pre-specified 5' and 3' primer pairs are also reportable.

AKT2	COL1A1	ERG	EWSR1	FLT3	MYB	NR4A3	NUTM1	PIK3CA	PTEN	ROS1	STAT6
ALK	CSF1	ESR1	FGFR1	FUS	MYBL1	NRG1	PAX3	PLAG1	RAD51B	RSPO2	TERT
AXL	EGFR	ETV1	FGFR2	JAK2	NF1	NTRK1	PAX7	PPARG	RAF1	RSPO3	TFE3
BRAF	ERBB2	ETV4	FGFR3	KRAS	NOTCH1	NTRK2	PDGFRA	PRKACA	RELA	SSX1	YWHA
CAMTA1	ERBB4	ETV5	FGR	MET	NOTCH4	NTRK3	PDGFRB	PRKACB	RET	SSX4	

Clinical Performance

The Strata Select test was developed and the performance characteristics determined by Strata Oncology. The test has neither been cleared nor approved by the U.S. Food and Drug Administration (FDA). The FDA has deemed that such clearance or approval is not necessary. Strata Oncology is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA) as qualified to perform high-complexity clinical laboratory testing. The Laboratory Director is Scott A. Tomlins, M.D., Ph.D.

Strata Select Test Description

The Strata Select test is a 437 gene, pan-solid tumor, next generation sequencing (NGS) assay for FFPE tumor tissue, performed on co-isolated DNA and RNA^{1,2}. In addition to simultaneous assessment of single-/multi-nucleotide variants (SNVs), splice site variants (SSVs), short (<40bp) insertions and deletions (indels), copy number alterations (CNAs; amplifications or deep deletions), microsatellite instability (MSI) status, gene fusions, and tumor mutation burden (TMB), Strata Select also reports an integrated Immunotherapy Response Score (IRS) by a Cox proportional hazard (CPH) model incorporating TMB with gene expression of four tumor/tumor microenvironment biomarkers (PD-L1 [CD274], PD-1 [PDCD1], ADAM12 and TOP2A; each normalized to the median of three housekeeping genes [HMBS, CIAO1 and EIF2B1]). Prioritized mutations, which include pre-defined mutations (activating SNVs, indels and SSVs for oncogenes; hot spot, loss of function SNVs, frame-shifting indels, and cancer-related pathogenic SNVs/SSVs for tumor suppressors) are reported separately from all other high-confidence somatic variants of unknown significance. Genes and alteration types assessed by the Strata Select test are shown in the DNA Genes and RNA Gene sections. The Strata Select test covers nearly the entire coding sequence for mutation reporting in 411 genes (median 96.1% coverage, IQR 90.7%-99.1%) with targeted coverage of 6 oncogenes; the specific regions not covered are available upon request. A negative result does not indicate that a gene is negative for any possible alteration, only the specific alterations assayed by the Strata Select test. The variant allele frequency (VAF) limit of detection for oncogenic mutations (SNVs or frame-preserving indels), other SNVs, indels, splice site variants, and single base indels at homopolymers from the bioinformatic pipeline are <1.1%-3.7%, 7.5%-8.3%, 4.7%-5.7%, 4.1%-5.4%, and 11.8%-14.9%, respectively. Orthogonally validated indels up to 15 bases were detected in the Strata Select validation, and well supported indels up to 82 bases have been detected and reported by Strata Select testing, however indel detection is impacted by the location in the covering amplicon(s). For gene fusions, only specific gene fusion partners are assayed for (e.g. EML4-ALK) so novel gene fusion partners will not be detected unless 5' and 3' primers are included in Strata Select. For copy number events, the cellularity adjusted whole copy number estimate is thresholded at 6 copies and the 90% confidence interval lower bound is thresholded at 5 copies for calling amplifications in known oncogenes. The copy number estimate is thresholded at 0.5 copies and the 90% confidence interval upper bound is thresholded at 1 copy for calling deep deletions (homozygous deletions, assuming diploid state) in known tumor suppressor genes. Estimated tumor content, variant allele frequencies, and copy number (with associated 90% confidence interval) are reported for informational purposes only. MSI status is determined by thresholding an MSI score derived from length variant allele counts observed at multiple microsatellite loci; specimens with an MSI score greater than 0.5 are considered microsatellite instability high (MSI-High). Tumor mutation burden includes non-coding (at highly characterized genomic loci) and coding, synonymous and non-synonymous, single and multi-nucleotide (two bases) variants present at variant allele frequency (VAF) > ¼ of molecularly-informed tumor content (TC); mutation rate per megabase (Mb) estimate and associated 90% confidence interval are calculated via the total number of positions with sufficient depth of coverage necessary for definitive assessment (maximum possible 1.7Mb). Quantitative TMB results are reported, along with TMB status (TMB-Low: <10 mutations per Mb; TMB-High: ≥10+ mutations per Mb). A complete list of all prioritized genomic variants and transcript annotations is available upon request. The tumor content (determined at the time of molecular review and signout) limit of detection for all Strata Select variant classes is ≥20%.

Strata Select assays for mutations in tumor tissue only, and therefore does not distinguish between somatic and germline mutations. Suspected germline variants with cancer relevance are highlighted and described in a "Germline Note" beneath the Pertinent Biomarker Summary in the Strata Oncology Testing Summary based on the Intermediate-permissive approach described by the ESMO Precision Medicine Working Group (PMID: 36529447), which recommend germline follow-up pan-tumor for Most/High-Actionable cancer susceptibility genes (CTNNA1 and NBN are considered High-Actionable by Strata Select) and germline follow-up on-tumor for Standard-Actionable genes. Per ESMO, germline analysis/follow-up for variants in APC, PTEN, RB1, TP53, CDKN2A and SMARCA4 should be restricted to tumors arising in those <30 years, and variants in these genes are not highlighted in the "Germline Note" but should be considered for analysis/follow-up as clinically indicated. Inclusion in "Germline Note" indicates recommended follow-up

analysis and/or confirmatory testing as clinically indicated, NOT that the alteration is definitively germline. Variants reported elsewhere may be true germline variants (including sub-gene deep deletions), but do not meet inclusion criteria for this Section; Strata Select testing is not inclusive of all known hereditary cancer genes and should not replace comprehensive genetic testing when clinically indicated.

Like TMB alone, IRS provides prognostic and predictive information to help inform pan-solid tumor systemic treatment decision making for PD-1 or PD-L1 (together PD-(L)1) monotherapy³. Feature selection for the IRS algorithm was performed by lasso-penalized CPH to predict time to next therapy (TTNT; a validated real world progression free survival treatment endpoint^{4,5}) in 648 anti-PD-1 (pembrolizumab) treated patients from 26 tumor types. Individual sample IRS is reported by the following model, with IRS ≥ 0.873 (displayed on 0-100 scale as ≥ 54) reported as IRS High, and IRS < 0.873 (displayed on 0-100 scale as < 54) reported as IRS Low: $\text{IRS} = 0.273758 * \text{TMB} + 0.112641 * \text{PD-1} + 0.061904 * \text{PD-L1} - 0.077011 * \text{TOP2A} - 0.057991 * \text{ADAM12}$.

In the independent 248 patient (24 tumor types) validation cohort of patients treated with non-pembrolizumab PD-1 (n=194 [78%]) or PD-L1 (n=54 [22%]) monotherapy, IRS High vs. IRS Low patients had significantly longer TTNT (median 23.1 vs. 10.2 months; adjusted hazard ratio [aHR] 0.52, p=0.003) and overall survival (OS; median 40.4 vs. 21.4 months; aHR 0.49, p=0.005) when adjusted for age, gender, tumor type, PD-1/PD-L1 therapy, and line of therapy. Neither median TTNT nor OS significantly differed between IRS High/TMB High vs. IRS High/TMB Low patients (TTNT 21.0 vs. 28.2 months, p=0.31; median OS 40.4 months vs. Not Reached, p=0.53), confirming performance of IRS in both TMB High and TMB Low patients. Across 3,184 patients (23 tumor types) treated with a non-PD-(L)1 or CTLA4 containing first line systemic therapy, IRS status was not a significant predictor of TTNT (aHR 1.05, p=0.45), confirming the predictive nature of IRS.

In a second 352 patient anti-PD-(L)1 monotherapy validation cohort (31 tumor types; 82% treated with pembrolizumab), IRS-High vs. IRS-Low patients had significantly longer rwPFS (median 15.1 vs. 3.8 months, aHR 0.41, p<0.0001) and OS (41.0 vs. 11.7 months, aHR 0.47, p=0.0002). IRS significantly improved CPH associations with rwPFS and OS beyond microsatellite instability (MSI)/TMB status alone. In a 189 patient (10 tumor types) PD-L1 IHC comparison cohort, IRS, but not PD-L1 IHC nor TMB, was significantly associated with anti-PD-L1 rwPFS. In a 1,229 treatment-line cohort (from five relevant tumor types), rwPFS did not significantly differ in IRS-High patients treated with anti-PD-(L)1 vs. anti-PD-(L)1 + chemotherapy⁶. IRS associations were consistent across subgroups, including both Europeans and non-Europeans. IRS has been subsequently validated in additional studies under peer review.

IRS is not valid nor reported in samples failing either the TMB or gene expression component QC metrics, or in samples with <20% tumor content. IRS is not valid in patients who have already received anti-PD-(L)1 or CTLA4 therapy. We recommend submission of a current tissue specimen for the most relevant Strata Select results, particularly if the patient has been treated with targeted and/or hormonal therapy, or the tumor is suspected to have undergone transformation to small cell carcinoma.

Treatment Decisions

The selection of any treatment or clinical trial suggested by a biomarker resides within the discretion and judgment of the treating physician and patient. In the Strata Oncology Testing Summary, Strata Select identified biomarkers and relevant treatment associations are shown and described in the 'Therapy Associations' section based on the final reported tumor type using the following levels of evidence as determined by Strata: Level 1, FDA-recognized predictive biomarker in this indication; Level 2, NCCN or other professional guideline-recognized predictive biomarker in this indication; Level 3, Compelling predictive clinical evidence in this indication; Level 4, Compelling predictive biologic evidence in this indication. The biomarker associated benefit prediction ("Benefit", "Less Benefit", "Not indicated") is shown, with "Less Benefit" indicating less predicted clinical benefit compared to unselected patients. For tumor types where PD-(L)1 monotherapy is considered Level 1 or 2 evidence in the absence of a biomarker (or by PD-L1 immunohistochemistry), that level of evidence is maintained if the combination of MSI/TMB/IRS supports PD-(L)1 monotherapy benefit. Line of therapy (first, second, later, maintenance) are shown by superscript (* indicates a NCCN preferred therapy in that line). Listed treatments and levels of evidence may not be applicable to the patient's specific cancer based on incorrectly reported tumor type (most commonly due to information not provided in the submitted pathology report), prior therapies, impact of other biomarkers not assessed by Strata testing, and specific indications requiring combination with non-targeted therapies. Decisions on patient care should be based on the independent medical judgment of the treating physician based upon all available clinical info and should not be based solely on the individual Strata tests and information contained in the Strata Oncology Testing Summary.

No Warranty or Guarantee of Clinical Benefit

This report does not make any promise or guarantee that a particular therapy or clinical trial will be effective or helpful in the treatment of disease in any patient.

References

1. Tomlins SA, Hovelson DH, Harms P, et al: Development and Validation of StrataNGS, a Multiplex PCR, Semiconductor Sequencing-Based Comprehensive Genomic Profiling Test. *J Mol Diagn* 23:1515-1533, 2021
2. Tomlins SA, Hovelson DH, Suga JM, et al: Real-World Performance of a Comprehensive Genomic Profiling Test Optimized for Small Tumor Samples. *JCO Precis Oncol* 5, 2021
3. Tomlins SA, Khazanov N, Bulen BJ, et al: Development and Validation of an Integrative Pan-Solid Tumor Predictor of PD-1/PD-L1 Blockade Benefit. *Communications Medicine*, 7;3(1):14, 2023
4. Kehl KL, Riely GJ, Lepisto EM, et al: Correlation Between Surrogate End Points and Overall Survival in a Multi-institutional Clinicogenomic Cohort of Patients With Non-Small Cell Lung or Colorectal Cancer. *JAMA Netw Open* 4:e2117547, 2021
5. Quintanilha JCF, Graf RP, Fisher VA, et al: Comparative Effectiveness of Immune Checkpoint Inhibitors vs Chemotherapy in Patients With Metastatic Colorectal Cancer With Measures of Microsatellite Instability, Mismatch Repair, or Tumor Mutational Burden. *JAMA Netw Open* 6(1): e2252244, 2023
6. Bulen BJ, Khazanov NA, Hovelson DH, et al: Validation of Immunotherapy Response Score as Predictive of Pan-solid Tumor Anti-PD-1/PD-L1 Benefit. *Cancer Research Communications*, 3(7), 2023

Test Version

Laboratory workflow version: 4

Bioinformatics pipelines version: 4.10

CPT code: 81479

Test code: Z01Z0

Patient MRN ---	Patient Name ---, ---	Birth Date ---	Sex Female	Strata Case ---
Client Specimen ID ---	Specimen Site Lung, RII	Part ---	Date of Collection ---	Date Received ---
Client ---	Ordering Physician ---			

- Molecular Diagnosis and Tissue Specimen Information** Determined at time of testing

Cancer Type	Tumor Content
Breast Cancer	80%
Cancer Subtype	Surface Area
Breast Invasive Carcinoma, NOS	5mm ²
Receptor Status	
ER: Positive, PR: Negative, HER2: Low, ERBB2 copy number: 2.2	

- Antibody Drug Conjugate Treatment Response Scores**

Biomarker	Biomarker	Biomarker
FOLR1 TRS High: 91	LIV-1 TRS High: 95	Nectin-4 TRS High: 95
HER2 TRS High: 98	MET TRS Low: 45	TF TRS High: 93
HER3 TRS High: 99	NaPi2b TRS High: 94	Trop2 TRS High: 97

- Angiogenesis Inhibitor Treatment Response Score**

Angio TRS Low: 41

- Gene Expression Biomarker Results**

Biomarker	Biomarker	Biomarker	Biomarker
AR High: 15.6 Percentile rank: 97.5%	DKK1 Low: 6.1 Percentile rank: 12.3%	FOLR1 Low: 10.6 Percentile rank: 58.9%	LAG3 Low: 9.6 Percentile rank: 39.9%
AXL High: 10.2 Percentile rank: 56.0%	DLL3 Low: 6.7 Percentile rank: 59.7%	GUCY2C Low: 6.9 Percentile rank: 46.6%	LY75 (CD205) Low: 9.3 Percentile rank: 36.0%
NCAM1 (CD56) Low: 10.8 Percentile rank: 79.0%	EPHA2 Low: 6.8 Percentile rank: 5.9%	ERBB2 (HER2) Low: 11.7 Percentile rank: 93.0%	MET Low: 5.1 Percentile rank: 5.2%
CLDN18.2 Low: 1.3 Percentile rank: 18.0%	ESR1 (ER) Positive: 14.6 Percentile rank: 90.3%	EPAS1 (HIF2A) Low: 9.4 Percentile rank: 29.6%	CTAG1B (NY-ESO-1) Low: 3.3 Percentile rank: 24.0%
	FGF19 Low: 2.8 Percentile rank: 44.3%	IL2RA Low: 9.3 Percentile rank: 34.1%	

Biomarker

SLC34A2 (NaPi2b) Low:
12.4

Percentile rank: 68.7%

NECTIN4 (Nectin-4)
High: 11.5

Percentile rank: 81.4%

Biomarker

PGR (PR) Negative: 9.7

Percentile rank: 47.4%

FOLH1 (PSMA) Low: 8.9

Percentile rank: 26.9%

ROR2 High: 12.2

Percentile rank: 91.6%

Biomarker

TIGIT High: 10.1

Percentile rank: 52.1%

TACSTD2 (Trop2) High:
12.7

Percentile rank: 95.6%

• Additional Gene Expression Results

Biomarker

ALPG: 4.4

Pan-tumor percentile rank: 29.0%

EGFR: 8.0

Pan-tumor percentile rank: 14.4%

FGFR1: 9.4

Pan-tumor percentile rank: 34.9%

Biomarker

FGFR2: 12.6

Pan-tumor percentile rank: 93.7%

FGFR3: 12.3

Pan-tumor percentile rank: 86.1%

IGF1R: 11.5

Pan-tumor percentile rank: 91.8%

Biomarker

Proliferation score: 9.7

Pan-tumor percentile rank: 41.9%

• Notes

- The specimen yielded DNA concentration less than 1ng/uL.

Rationale

The Strata RNA Supplement is a laboratory-developed test performed in the Strata Oncology CLIA-certified laboratory. Selected gene expression results are reported from an analytically and clinically validated gene expression panel. The reported thresholds for determining low vs. high expression of each biomarker, as well as associations with IHC, have not been prospectively validated nor prospectively shown to associate with benefit or lack of benefit from any listed associated therapy. Clinical utility has not been established for this test. These test results are provided as they may be useful for correlating with treatment response to approved or investigational therapies, as well as other clinical, molecular, or pathological findings. Biomarker results may also confer eligibility for clinical trials.

Clinical Performance

The Strata RNA supplement test was developed and the performance characteristics determined by Strata Oncology. The test has neither been cleared nor approved by the U.S. Food and Drug Administration (FDA). The FDA has deemed that such clearance or approval is not necessary. Strata Oncology is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA) as qualified to perform high-complexity clinical laboratory testing. The Laboratory Director is Scott A. Tomlins, M.D., Ph.D

Strata RNA Supplement Test Description

The Strata RNA supplement generates quantitative gene expression data by multiplex PCR based next generation sequencing (NGS) in parallel with the RNA component of Strata Select testing (for gene fusions and Immunotherapy Response Score gene expression components)¹. The current version of Strata RNA supplement reports 31 individual target gene biomarkers and 11 composite gene expression biomarkers (9 antibody drug conjugate (ADC) Treatment Response Scores, Angio Treatment Response Score and Proliferation Score).

Strata RNA supplement accuracy was validated by an expression survey of all individually reported targets, individual target components of composite biomarkers, and composite biomarkers vs. a reference set of pan-solid tumor gene expression, as well as by representative validation vs. quantitative reverse transcription PCR (qRT-PCR) and/or immunohistochemistry (IHC).

Individual target expression scores are determined in log₂ units, representing target gene expression (normalized to the median expression of three pan-solid tumor stable housekeeping genes [HMBS, CIAO1 and EIF2B1]), then scaled to the distribution observed in a common control (in normalized reads per million [nRPM]), and quantile normalized per fixed, RNA panel/laboratory workflow. All individual targets and composite biomarkers are scaled such that the median score is 10.0 across the observed pan solid tumor range (>30,000 tumors at the time of database lock), with each unit increase representing a doubling from the median. In addition to an expression score, the pan-solid tumor percentile rank is also reported. Angio (multiple angiogenesis related target genes), proliferation (TOP2A and UBE2C) and HER2 (two distinct HER2 amplicons) composite biomarkers are determined by the average of the individual, quantile normalized, target gene values. The tumor content limit of detection for all individually reported and composite gene expression biomarkers is 20%.

In two separate validation cohorts of 141 and 218 evaluable breast cancer tissue samples, pre-specified Strata RNA supplement ER Positive (>13.684) vs. Negative (<11.934) status had 98.0% & 98.8% sensitivity and 100% & 98.2% specificity vs. ER status by clinical IHC (ER IHC >10% as Positive and IHC 0% as Negative; IHC 1-10% were excluded); samples with ER expression 11.934-13.684 are reported as ER Low and are considered inconclusive for ER (and hormone receptor) status and therapy associations, with deferral to clinical IHC in such cases recommended. In two separate validation cohorts of 122 and 174 evaluable breast cancer tissue samples, pre-specified Strata RNA supplement PR gene expression Positive (>13.2395) vs. Negative (<12.0) status had 95.7% & 93.4% sensitivity and 100% & 96.9% specificity vs. PR status by clinical IHC (PR IHC >10% as Positive and PR IHC 0% as Negative; PR IHC 1-10% were excluded); samples with PR expression 12.0-13.2395 are reported as PR Low and are considered inconclusive for PR status, with deferral to clinical IHC in such cases recommended.

Four HER2 categories are reported by the Strata RNA supplement: Negative (score ≤10.5935; breast 0-1+ IHC equivalent), Low (score 10.5935 to <12.50431; 1-2+ IHC equivalent), Moderate (score 12.50431-≤13.70431; 2+ IHC equivalent), Positive (score >13.70431; 3+ IHC equivalent). In two separate validation cohorts of 216 and 191 evaluable breast cancer tissue samples, pre-specified Strata

Strata RNA Supplement

RNA supplement HER2 gene expression Positive vs. Negative or Low status had 80.0% & 93.8% sensitivity and 100.0% & 100.0% specificity vs. HER2 status by clinical IHC (HER2 IHC 3+ as Positive and IHC 0-1+ as Negative; IHC 2+ were excluded). HER2 amplification status (by Strata Select) is used to make first line HER2 targeted therapy associations in Strata RNA supplement HER2 Moderate samples. Given the later line association of trastuzumab deruxtecan in HER2 IHC 1+ and 2+ (FISH/ISH negative) breast cancer, and the poor reproducibility of IHC distinction between 0+ and 1+ in routine practice², the Strata RNA supplement HER2 Negative vs. Low expression threshold was set at the lower 25th percentile of IHC 1-2+ (non-ERBB2 amplified) samples in a 139 sample training cohort (where 39% of 0+ IHC samples were above this threshold) and applied to the 0+ IHC samples (n=124 and 91) in the two validation cohorts. In the two validation cohorts, 34% and 24% of 0+ IHC samples were above the Strata RNA supplement HER2 Negative threshold.

Antibody Drug Conjugate Treatment Response Scores for FOLR1, HER2, HER3, LIV-1, c-Met, NaPi2b, Nectin-4, TF, and TROP-2 are RNA expression based biomarkers integrating ADC target, proliferation, and cell adhesion. The TRS model better predicted objective response rates (ORR) across ADCs and tumor types than RNA expression of ADC target alone in a training cohort (n=16 observed ORRs [from 7 tumor types and 8 ADCs]) and a validation cohort (n=11 observed ORRs [from 9 tumor types and 3 ADCs]).

The Angiogenesis Treatment Response Score is a multi-gene expression algorithm that integrates multiple angiogenesis related target genes chosen based on co-expression patterns across VEGFR TKI responsive and resistant tumor types. The High/Low threshold (11.613), was set at the 42nd percentile of Strata tested clear cell renal cell carcinomas. In a validation cohort of 86 renal cell carcinomas (66 clear cell and 20 non-clear cell) treated with single agent VEGFR TKI systemic therapy, Angio High samples had significantly longer real-world progression free survival (rwPFS; by time to next therapy) vs. Angio Low samples when controlling for patient age, gender, line of systemic therapy, clear cell vs. non-clear cell histology, ISUP/WHO nuclear grade (incorporating sarcomatoid features), and TKI type (median rwPFS 15.8 vs. 5.6 months, adjusted hazard ratio 0.46, p=0.012).

In a validation cohort of 39 evaluate evaluable high grade serous ovarian/fallopian tube/primary peritoneal cancer tissue samples, pre-specified StrataEXP FOLR1 High (≥ 15.5) vs. StrataEXP FOLR1 Low (< 15.5) status had 96.4% sensitivity and 100% specificity vs. the mirvetuximab soravtansine companion diagnostic FOLR1 IHC assay (VENTANA FOLR1 RxDx Assay; RxDx IHC $\geq 75\%$ 2-3+ positive as Positive and $< 50\%$ 2-3+ positive as Negative; IHC 50-70% 2-3+ samples were excluded based on poor repeatability and reproducibility of this group in the FDA validation (https://www.accessdata.fda.gov/cdrh_docs/pdf22/P220006B.pdf). Of the 9 RxDx IHC 50-70% 2-3+ positive samples, 8 were StrataEXP FOLR1 High.

For remaining individual expression biomarkers, high/low biomarker thresholds were set based on pan-solid tumor or specific tumor type expression distributions observed in Strata RNA supplement tested tumor samples. Some biomarkers are reported without a threshold, and are equivalent to genomic variants of uncertain significance reported by Strata Select. Biomarker results may also confer eligibility for clinical trials. Strata RNA supplement results are provided as they may be useful for correlating with treatment response to associated or non-associated therapies or other clinical, molecular, or pathological findings. No Strata RNA supplement reported individual or composite biomarker thresholds have been shown to prospectively associate with benefit or lack of benefit from any therapy. When ordered as part of a Strata Oncology sponsored clinical trial or used to facilitate enrollment into a Strata Oncology sponsored clinical trial, and where applicable pursuant to the trial protocol, this test shall be considered subject to the following disclaimer: For Investigational Use Only. The Performance Characteristics of this Product Have Not Been Established.

No Warranty or Guarantee of Clinical Benefit

This Summary does not make any promise or guarantee that a particular therapy or clinical trial will be effective or helpful in the treatment of disease in any patient.

Treatment Decisions

The selection of any treatment or clinical trial associated with a biomarker resides within the discretion and judgment of the treating physician and patient. In the Strata Oncology Testing Summary, Strata RNA supplement identified biomarkers and relevant treatment associations are shown and described in the 'Therapy Associations' section based on the final reported tumor type using the following levels of evidence as determined by Strata: Level 1, FDA-recognized predictive biomarker in this indication; Level 2, NCCN or other professional guideline-recognized predictive biomarker in this indication; Level 3, Compelling predictive clinical evidence in this

indication; Level 4, Compelling predictive biologic evidence in this indication. The biomarker associated benefit prediction ("Benefit", "Less Benefit", "Not indicated") is shown, with "Less Benefit" indicating less predicted clinical benefit compared to unselected patients. Line of therapy (first, second, later, maintenance) are shown by superscript (* indicates a NCCN preferred therapy in that line). Listed treatments and levels of evidence may not be applicable to the patient's specific cancer based on incorrectly reported tumor type (most commonly due to information not provided in the submitted pathology report), prior therapies, impact of other biomarkers not assessed by Strata testing, and specific indications requiring combination with non-targeted therapies. Whether expression profiles of tumors are maintained after progression or therapy is unclear, and we recommend submission of a current tissue specimen for the most relevant Strata RNA supplement results. Decisions on patient care should be based on the independent medical judgment of the treating physician based upon all available clinical info and should not be based solely on the individual Strata tests and information contained in the Strata Oncology Testing Summary.

References

1. Tomlins SA, Khazanov N, Bulen BJ, et al: Development and Validation of an Integrative Pan-Solid Tumor Predictor of PD-1/PD-L1 Blockade Benefit. *Communications Medicine*, 7;3(1):14, 2023
2. Fernandez AI, Liu M, Bellizzi A, et al: Examination of Low ERBB2 Protein Expression in Breast Cancer Tissue. *JAMA Oncol* 8(4): 1-4, 2022

Test Version

Laboratory workflow version: 4

Bioinformatics pipelines version: 4.10